Project No.
Book No.
TITLE Deep Vent / GAPDH / dif primers

96 11/16/94

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Purpose: Since GAPDH - PCR worked with 3' Thiol primers attempted the same amplification with other available primers, under same conditions.

- Deepvent buffer
- 200 pg Template
- 200 μM dNTP
- 1 μM primers
- did just one of each:
  - # 2697 & 2696
- enzyme at 1 U and 0.5 U
- Mg at 2, 3, 4 and 6 mM

primers

no dU - Lac FWD & Lac Reverse (100 μM)
" " " 3'-1 PPT
+dU " " " (100 μM)

- each primer set - 10x RXN were made.

	Regular (17-20 = 0.5 U; 21-24 = 1.0 U)	dU (33-36 = 0.5 U; 37-40 = 1.0 U)	3'-1 PPT	
10x buffer	50	50	50	25
dNTP	10	10	10	
primer 1	5	50	20	1
2	5	50	20	
Template	20 (100 pg/λ)	20	200	29
H_2O	260	270	330	
	450 → 45 μl/RX ←	450	← 450	

added 5μl of Mg dilution

	2	3	4	6	mM	
	1	1	1	1		
μl	0	0.5	1	2	(100 mM)	10x
	5	4.5	4	3	H_2O	

- enzyme added individually 0.25 μl for 0.5 U
  0.5 μl for 1 U

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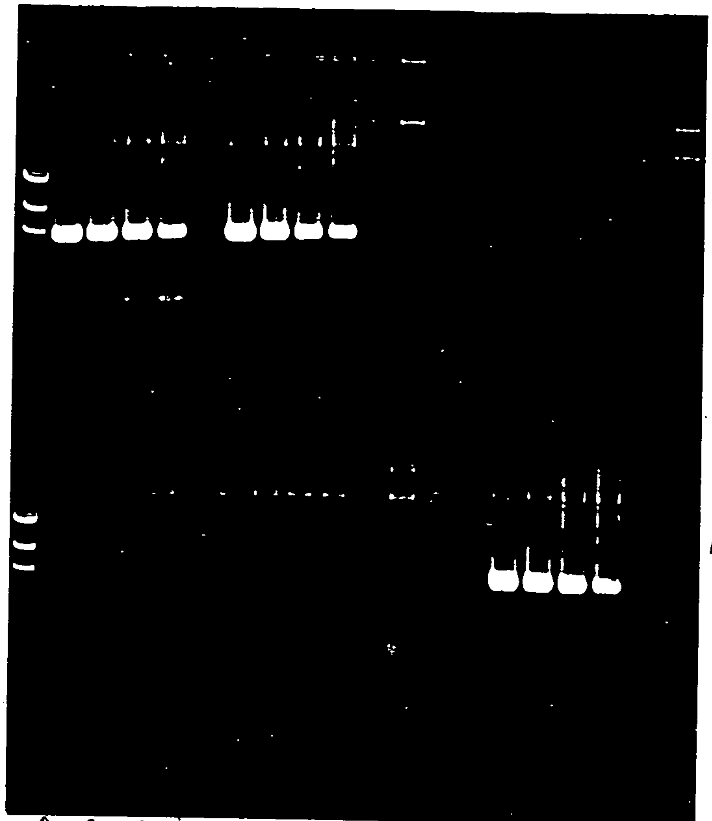
Witnessed & Understood by me,	Date	Invented by	Dated
	11/21/94	Recorded by K. Abramson	11/16/94

ag N . Regular 3'-1 PPT

0.5U



1U

2 3 4 6 2 3 4 6 mwt Mg
..il 0.5 1U

Result:

- Regular - unmodified, worked, so couldn't say 3'-1 PPT is better than unmodified!
- dU certainly has problem with Deep Vent.
- With the amount of product seen with 1U don't know why there is no product with 0.5U & 3'-1 PPT primers.

Conc of template - ? Contamination - ? - what are these bands on the top.

- Template / primer - no enzyme controls also have them ?

dU primer

Vent +/- exo didn't discriminate between modified and unmodified

" with dU in earlier times pcr & 500 bp Lac 2 never got amplified.

- Samples discarded 12/19/94

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ed & Understood by m , Date 11/28/94 Invented by Dat 11/18/94
Recorded by K. Sitaraman